than radical initiated within the bilayer core. However for lipids where vitamin E resides slightly lower(glycerol backbone) we observe comparable antioxidant activity against both water borne and hydrocarbon borne oxidants. Thus showing lipid species can modulate the location of vitamin E's activity.

1282-Pos Board B174
Exploring the Sequence Determinants of Spontaneous Membrane-Translocating Peptides
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The development of cell penetrating peptides (CPPs) has long promised to enable the delivery of a wide variety of polar compounds into cells. Yet the lack of tools for engineering and designing such peptides creates a bottleneck in the discovery pipeline. In addition, a consensus raised from mounting studies favors a mechanism that requires one or more types of energy-dependent endocytotic pathways for cellular uptake of most CPPs, which significantly limits their applications. The goal of this work was to learn to engineer spontaneous membrane translocating peptides (SMTPs) that move across lipid bilayers and cellular membranes in an energy-independent manner. A previous orthogonal screen of a synthetic peptide library (N=10,368) for SMTPs revealed a conserved 9-residue motif (PLI[L/Y]LRLLR) in a family of 12-residue SMTPs that translocate rapidly without causing any bilayer destabilization. In this work, we used one of these SMTPs, PLIYLRLLRGQF, as the template for rational iterations of SMTPs designed to reveal the sequence determinants of spontaneous translocation. Translocation of the variants was measured in large unilamellar vesicles (LUVs) containing entrapped protease. We also measured vesicle permeabilization caused by each of these analogs. For each variant, the rate of peptide translocation and its effect on membrane permeability is discussed. Our results shed light on the determinants of spontaneous translocation, which may allow for the discovery and engineering of potent SMTPs to overcome the membrane barrier for drugs delivery.

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Exploring Amphotericin B-Membrane Interactions: Free Energy Simulations
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Amphotericin B (AmB) is a membrane-active polyene antibiotic used to treat serious fungal infections. Biological action of AmB is due to the formation of transmembrane channels. Membrane sterols are known to be crucial for the AmB antifungal activity, i.e. AmB is more active against fungal cell membranes containing ergosterol than against the mammalian membranes with cholesterol.
We explored molecular determinants of AmB selectivity for ergosterol-containing membranes using computational methods. By means of molecular dynamics simulations we studied various aspects of the interactions between AmB and lipid bilayers of different composition (containing or not 30% of ergosterol or cholesterol). More precisely, we examined (1) AmB insertion into a membrane, (2) changing tilt angle between AmB and the bilayer plane (for AmB embedded in a membrane) as well as (3) AmB dimerization in a membrane. To provide a thermodynamic description of these processes, we calculated the free energy profiles describing each of them in the three different membrane systems. The results indicate that at low, chemotherapeutically relevant concentrations of AmB at which the antibiotic expresses its channel-forming activity, AmB is mostly monomeric in ergosterol-containing membranes and it exists predominantly as a dimer in cholesterol-containing (and sterol-free) ones. We also show that compared to the other two studied membranes, it is the most favorable for AmB to insert the ergosterol-containing bilayer. The differences in the behavior patterns of AmB in bilayers of different composition are mainly of energetic origin. From the free energy profiles for (2) and (3) we also determine the most preferred location and orientation of AmB within the studied bilayers.

1284-Pos Board B176
Conductance of Ideally Cation-Selective Ion Channel Depends on Anion Type
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Gramicidin A (gA) is a cation selective ion channel that has been used in many biophysical studies of lipid bilayers, in particular for investigations of lipid-protein interactions [1, 2] and membrane electrostatics [3]. In addition, it was found that ionic interactions with neutral lipid membranes also affect the kinetics of gA channels [4]. Here we report measurements of gA ion-channels for a series of sodium and potassium salts that show an *anion*-dependence of gA conductance. We find that gA conductance varies significantly with the anion type with ClO_4 and SCN producing distinctly larger conductance values than Cl, F, and H_2PO_4. These results can provide new insights into ion-lipid membrane interactions and ion channel functions in general. [1] Andersen et al., Annu. Rev. Biophys. Biomol. Struct., 1996, [2] Lunbaek et al., PNAS 2010, [3] Rostovtseva et al. Biophys. J. 1998, [4] Rostovtseva et al. Biophys. J. 2008.

1285-Pos Board B177
Nanoscopic Cell Membrane and Pore Profiles Combining Molecular Dynamics and a 3D Electromagnetic Tool
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Nanosecond, megavolt-per-meter electric pulses applied to biological cells can target subcellular structures with minimal loss of plasma membrane integrity, opening up new perspectives for intracellular manipulations. Experimentally observed effects of intense nanopulses include intracellular calcium release, externalization of phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, and non-thermal cell death by apoptosis. Molecular dynamics (MD) simulations have shown that PS re-distribution occurs after the electric-field-driven formation of nanometer-sized pores in the plasma membrane and is facilitated by electrophoresis of PS along the pore walls. Nanopulse-induced pore creation occurs on a nanosecond time scale, but the underlying molecular mechanisms are not yet clear. Experimental observations of the process of pore formation are challenging because of the time and spatial scales required.
In this study, we combine MD simulations and a quasi-static approach using a custom implementation of 3D finite-difference analysis to investigate the physical mechanisms of electropore creation. First, MD simulations of pore formation in phospholipid bilayers in external electric fields are performed at nanoscopic scale. From these simulations we extract the charge densities across the electroporated bilayer. Second, the charge densities are injected into a new, custom, quasi-static algorithm based on the Poisson equation. The software computes 3D nanoscopic profiles of the transmembrane potential, electric field, and electric field gradient. The goal of the two-step simulation is to establish whether and how electric field gradients, water and phospholipid head group dipole moments, and the site of initial water intrusion in pore initiation are correlated.

1286-Pos Board B178
Biophysical and Biological Behaviour of Ciprofloxacin and Ciprofloxacin Derivatives: A Route to Counteract Bacteria Resistance?
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Studies of bacterial membranes are fundamental in the understanding and counteracting of bacterial resistance. Direct attack on bacterial membranes is thought to be a way to counteract bacterial resistance mechanisms, which are mostly based on intracellular adaptations. Therefore, a current trend in antibiotic research is specifically targeting bacterial membranes, leading to permeation of the membrane, inducing the formation of membrane domains and ultimately leading to cell death. The fact that such effects are not observed in the interaction with mammalian cells illustrates the capacity that such antibiotics have to discriminate between bacterial and mammalian cells, most likely due to the differences in the lipid composition of the two types of cell membranes.
In this work we present fluorescence spectroscopy studies of the interaction of ciprofloxacin and phenanthroline/copper/ciprofloxacin complex with several lipidic mimetic systems. POPE/POPG (0.75:0.25), POPE/POPG/Cardiolipin (0.67:0.23:0.10), E. coli total lipid extract and DMPC liposomes were used and the results obtained point out to a very different behavior between ciprofloxacin and its copper complex. Preliminary results of the interaction of these compounds with OmpF proteoliposomes will also be presented. These results, together with biological data, suggest that the ciprofloxacin complex can be an alternative to counteract bacterial resistance to these antibiotics.
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